UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/049,825	02/19/2002	Wolfgang M. Franz	0690-0115P	6306

2292 7590 07/17/2003

BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747

EXAMINER
LI, QIAN J

ART UNIT	PAPER NUMBER
1632	

DATE MAILED: 07/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/049,825	FRANZ, WOLFGANG M.	
	Examiner	Art Unit	
	Q. Janice Li	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 May 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-29,32 and 33* is/are pending in the application.

4a) Of the above claim(s) *18-29 and 33* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17,32* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *19 February 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Election/Restrictions

Applicant's provisionally election with traverse of Group I, drawn to an expression cassette, and a method of using such for genetic alteration of pluripotent cells of a mammal, in Paper No. 7 is acknowledged. The traversal is on the ground(s) that careful reading of the claims should result in inclusion of additional claims into group I, because step (a) of the claims 18 and 19 is introducing the expression cassette into pluripotent cells as claimed in claim 30. The request has been carefully considered but not found persuasive because group I (claim 30, now renumbered new claim 32) and group II (claims 18, 19) are each drawn to a different method of using the expression cassette, i.e. genetically manipulating pluripotent cells or isolating a differentiated somatic cells. The method step of claim 30 stands alone, and has separate utility. 37 CFR 1.475 (b) states "an international or a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to **one** of the following combinations of categories: (1) A product and a process specially adapted for the manufacture of said product; or (**2**) **A** product and **a** process of use of said product. 37 CFR 1.475 (b) does not provide for more than one process as a combination of the invention. Moreover, claims 1 and 16, but not claims 18 and 19, are anticipated by *Segre et al, Reppert et al*, and *Harlan et al* (See § 102). Consequently, the special technical feature that links claims 1, 16, 18, and 19, does not

provide a contribution over the prior art as a whole, so unity of invention is lacking and restriction is appropriate.

Therefore, it is maintained that these inventions are distinct due to their divergent subject matter and are thus, separately classified and searched. The requirement is still deemed proper and is therefore made **FINAL**.

Please note that after a final requirement for restriction, the Applicants, in addition to making any response due on the remainder of the action, may petition the Commissioner to review the requirement. Petition may be deferred until after final action on or allowance of claims to the invention elected, but must be filed not later than appeal. A petition will not be considered if reconsideration of the requirement was not requested. (See § 1.181.).

Claims 1-29, 32, and 33 are pending, claims 4, 7, 10, 12, 13, 15-17, 21-23, and 27-29 have been amended, claims 30 and 31 have been canceled, and claims 32 and 33 are newly submitted. Claims 18-29, and 33 are withdrawn from further consideration by the Examiner, 37 CFR 1.142(b), as being drawn to non-elected inventions. Claims 1-17 and 32 are under current examination.

Claim Objections

The numbering of claims is not in accordance with 37 CFR 1.126 which requires the original numbering of the claims to be preserved throughout the prosecution. When claims are canceled, the remaining claims must not be renumbered. When new claims

are presented, they must be numbered consecutively beginning with the number next following the highest numbered claims previously presented (whether entered or not). Specifically, the newly submitted claims 33 and 34 are not numbered consecutively beginning with the number next following the highest numbered claims previously presented. Misnumbered claims 33 and 34 have been renumbered as claims 32 and 33.

Claims 1-17 are objected to because an article should precede "expression cassette" or "vector".

Claim 1 is objected to because the claim recitation, "non-immunogenic receptor" does not place a meaningful limitation on the claimed invention, the expression cassette. The specification teaches that the term refers to a receptor that is non-immunogenic to the host organism. However, since a certain receptor may be immunogenic to one host but not the other, and the expression cassette is not limited to be used in a specific host, thus, any receptor would meet claim limitation.

Claim 2 is objected to because the recitations in a) and c) do not place a meaningful limitation on the claimed invention. The claims are product claim, not limited to the use in a specific mammal, the receptor could be alien to one species but not the other, thus, virtually any receptor not expressed in the stem cells would meet claim limitation. Further, virtually all cells in a mammal is organ-specific or tissue specific, and the cassette could be used for modifying any other cell genetically, thus, any receptor on the surface of a non-stem cell would meet claim limitation.

Specification

The specification is objected to because the section of "Brief Description of the Drawings" is missing from the specification (See M.P.E.P. 608.01(f)). Appropriate correction is required.

Claim Rejections - 35 USC § 101

Claim 32 is rejected under 35 U.S.C. 101 because the claimed invention encompasses non-statutory subject matter. Claim 32 is drawn to a method for genetically modify pluripotent precursor cells of a mammal, which encompasses human embryonic stem cells, and is drawn to non-statutory subject matter.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 32 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for transforming pluripotent precursor cells of a mammal *in vitro*, does not reasonably provide enablement for doing so *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims.

The factors to be considered when determining whether the disclosure satisfies the enablement requirements and whether undue experimentation would be required to

make and use the claimed invention are summarized in *In re Wands*, (858 F2d 731, 737, 8 USPQ 2d 1400, 1404, (Fed Cir.1988)). These factors include but are not limited to the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, the breadth of the claims, and amount of direction provided. The factors most relevant to this rejection are the scope of the claims relative to the state of the art and the levels of the skilled in the art, and whether sufficient amount of direction or guidance are provided in the specification to enable one of skill in the art to practice the claimed invention.

Claim 32 is directed to a method for genetically modify pluripotent precursor cells of a mammal, and broadly encompasses *in vivo* gene therapy of pluripotent stem cells with any type of expression vector by any routes of administration using any tissue-specific promoter for delivering any therapeutic protein. However, the specification is silent with regard to in vivo manipulation of pluripotent precursor cells.

In view of the state of the art, it is still under development in the gene therapy and stem cell therapy fields. Claimed method requires gene targeting specifically to pluripotent stem cells. However, the specification fails to teach how to specifically targeting pluripotent stem cells *in vivo*. While progress has been made in recent years for gene transfer *in vivo*, vector targeting to desired cells *in vivo* continues to be unpredictable and inefficient as supported by numerous teachings available in the art. For example, *Deonarain* (1998, Expert Opin. Ther. Pat., Vol. 8, pages 53-69) indicates that one of the biggest problems hampering successful gene therapy is the "ABILITY TO TARGET A GENE TO A SIGNIFICANT POPULATION OF CELLS AND EXPRESS IT AT ADEQUATE LEVELS FOR

A LONG ENOUGH PERIOD OF TIME" (page 53, first paragraph). Deonarain reference gives high hope to targeted gene delivery, but the discussed strategies are still under investigation, and at the time, they were much less efficient than viral gene delivery (Conclusion), Moreover, transgene expression in vivo is generally inefficient, *Russell et al* (Nat Genet 1998 Apr; 18:325-30) review "IT IS CURRENTLY POSSIBLE TO INTRODUCE DEFINED MUTATIONS INTO MAMMALIAN CHROMOSOMES BY GENE TARGETING USING TRANSFECTION (ELECTROPORATION OR CALCIUM-PHOSPHATE PRECIPITATION) OR MICROINJECTION METHODS. TRANSFECTION TECHNIQUES USUALLY PRODUCE HOMOLOGOUS RECOMBINATION EVENTS IN ONLY A SMALL FRACTION OF THE TOTAL CELL POPULATION. CHROMOSOMAL GENE-TARGETING EXPERIMENTS HAVE BEEN PERFORMED WITH RETROVIRAL AND ADENOVIRAL VECTORS, BUT THE RECOMBINATION RATES WERE NOT SIGNIFICANTLY HIGHER THAN THOSE OBTAINED BY TRANSFECTION. (2nd paragraph, page 325). *Boucher et al* (J Clin Invest 1999 Feb; 103:441-5) review that host cell resistance to foreign gene is another difficulty for successful in vivo gene transfer. "DESPITE AN IMPRESSIVE AMOUNT OF RESEARCH IN THIS AREA, THERE IS LITTLE EVIDENCE TO SUGGEST THAT AN EFFECTIVE GENE-TRANSFER APPROACH FOR THE TREATMENT OF CF LUNG DISEASE IS IMMINENT. THE INABILITY TO PRODUCE SUCH A THERAPY REFLECTS IN PART THE LEARNING CURVE WITH RESPECT TO VECTOR TECHNOLOGY AND THE FAILURE TO APPRECIATE THE CAPACITY OF THE AIRWAY EPITHELIAL CELLS TO DEFEND THEMSELVES AGAINST THE PENETRATION BY MOIETIES, INCLUDING GENE-THERAPY VECTORS, FROM THE OUTSIDE WORLD." *Zink et al* (Gene Ther Mol Biol 2001 Jan;6:1-24) teach the reasons why the transgene would fail to achieve the expected effect in vivo, and indicated that in addition to the interaction of transcription factors with specific DNA elements, the transcription of mammalian genes and transgenes integrated into mammalian genomes is regulated at the levels of chromatin

structure and nuclear architecture, "TRANSCRIPTIONAL REGULATION OF INTERGRATING GENE THERAPY VECTORS IS ONLY WELL INVESTIGATED AT THE MOLECULAR LEVEL, FEW DATA EXIST REGARDING THE INVOLVEMENT OF CHROMATIN STRUCTURE, AND VIRTUALL NOTHING IS KNOWN ABOUT THE INVOLVEMENT OF NUCLEAR CHROMOSOME- AND GENOME ARCHITECTURE. THEREFORE, IT IS NOT SURPRISING THAT THE EXPRESSIONAL BEHAVIOR OF GENE THERAPY VECTORS AFTER INTEGRATION IS OFTEN UNPREDICTABLE AND DIFFICULT TO IMPROVE" (abstract). The specification is silent with regard to how to target the pluripotent progenitor cells *in vivo*, the rate of transfection *in vivo* and efficiency of gene expression in pluripotent precursor cells of various species of a mammal under either *in vivo* or *in vitro* circumstances.

The claims are drawn to using any naked polynucleotides and vectors. However, the specification fails to teach the type of vectors suitable for the purpose of the instant invention. *Robbins et al* (Pharmcol Ther 1998;80:35-47) teach that each type of vector system has its unique advantages and limitations, "RETROVIAL VECTORS CAN PERMANENTLY INTEGRATE INTO THE GENOME OF THE INFECTED CELL, BUT REQUIRE MITOTIC CELL DIVISION FOR TRANSDUCTION. ADENOVIRAL VECTORS CAN EFFICIENTLY DELIVER GENES TO A WIDE VARIETY OF DIVIDING AND NONDIVIDING CELL TYPES, BUT IMMUNE ELIMINATION OF INFECTED CELLS OFTEN LIMITS GENE EXPRESSION IN VIVO. HERPES SIMPLEX VIRUS CAN DELIVER LARGE AMOUNTS OF EXOGENOUS DNA; HOWEVER, CYTOTOXICITY AND MAINTENANCE OF TRANSGENE EXPRESSION REMAIN AS OBSTACLES. AAV ALSO INFECTS MANY NONDIVIDING AND DIVIDING CELL TYPES, BUT HAS LIMITED DNA CAPACITY" (abstract). *Robbins et al* go on to teach that non-viral vectors such as naked DNA and liposomes are inefficient in gene transfer to cell nucleus (Section 2, page 36). *Miller et al* (1995, FASEB J., Vol. 9, pages 190-199), acknowledge various vector system available in the art, then teach, "NO SINGLE DELIVERY SYSTEM IS LIKELY TO BE

UNIVERSALLY APPROPRIATE, FOR INSTANCE, THE REQUIREMENTS OF GENE THERAPY FOR CYSTIC FIBROSIS ARE GREATLY DIFFERENT FROM THOSE OF CANCER" (1st paragraph, page 190). "ONCE AGAIN, TARGETING AT THE LEVEL OF THE VECTOR HAS NOT YET BEEN PARTICULARLY WELL DEVELOPED; HENCE, LIPOSOME OR VIRAL-MEDIATED DELIVERY OF THE CFTR GENE TO AIRWAY EPITHELIAL CELLS OF CF PATIENTS HAS RELIED LARGELY ON THE LOCALIZED DELIVERY OF THE VECTORS DIRECTLY TO THE AFFECTED TISSUES" (1st paragraph, page 198) *Makrides et al* (Protein Exp Pur 1999;17:183-202) teach "THE CHOICE OF AN EXPRESSION SYSTEM FOR PRODUCTION OF RECOMBINANT PROTEINS DEPENDS ON MANY FACTORS, INCLUDING CELL GROWTH CHARACTERISTICS, EXPRESSION LEVELS, INTRACELLULAR AND EXTRACELLULAR EXPRESSION, POSTTRANSLATIONAL MODIFICATIONS AND BIOLOGICAL ACTIVITY OF THE PROTEIN OF INTEREST, AS WELL AS REGULATORY ISSUES AND ECONOMIC CONSIDERATIONS IN THE PRODUCTION OF THERAPEUTIC PROTEINS". *Verma et al* (Nature 1997;389;239-42) teach "THE USE OF VIRUSES IS A POWERFUL TECHNIQUE, BECAUSE MANY OF THEM HAVE EVOLVED A SPECIFIC MACHINERY TO DELIVER DNA TO CELLS. HOWEVER, HUMANS HAVE AN IMMUNE SYSTEM TO FIGHT OFF THE VIRUS, AND OUR ATTEMPTS TO DELIVER GENES IN VIRAL VECTORS HAVE BEEN CONFRONTED BY THESE HOST RESPONSE" (last paragraph of left column on page 239). The specification is silent with regard to the rate of transfection in vivo and efficiency of gene expression in pluripotent precursor cells of various species of a mammal under either in vivo or in vitro circumstances.

The claimed methods further require the use of tissue-specific or organ-specific promoters. *Miller et al* (Hum Gene Ther 1997 May;8:803-15) teach that "IT IS NOT UNCOMMON THAT CELLULAR *CIS*-ACTING SEQUENCES (TISSUE SPECIFIC) LOSE SOME OR ALL OF THEIR ABILITY TO RESTRICT EXPRESSION APPROPRIATELY WHEN PLACED IN THE CONTEXT OF A VIRAL

VECTOR...THE CELLULAR ENVIRONMENT MAY HAVE A STRONG EFFECT ON PROMOTER ACTIVITY...MANY OF THE PROBLEMS THAT ARE OBSERVED WHEN CELLULAR PROMOTERS ARE PUT IN VIRAL VECTORS ARE DUE TO THE POWERFUL ENDOGENOUS VIRAL TRANSCRIPTIONAL CONTROLS OVERRIDING THE CELLULAR SEQUENCE..." *Nettelbeck et al* (Gene Ther 2000 April; 16:174-181) teach "SOME OF THE RECENTLY DESCRIBED EXPERIMENTAL GENE THERAPY PROTOCOLS DO INDEED MAKE USE OF NATURAL TISSUE-SPECIFIC PROMOTERS, BUT IN MANY INSTANCES THESE PROMOTERS SUFFER FROM A LACK OF ACTIVITY, SPECIFICITY OR BOTH." (the paragraph bridging page 174-175). The specification fails to teach how to overcome the aforementioned difficulties in the art. It would have required undue experimentation for the skilled artisan intending to practice the instant invention.

Thus, it is evident that at the time of the invention, the gene therapy practitioner, while acknowledging the significant potential of gene therapy of pluripotent stem cells, still recognized that such therapy was neither routine nor accepted, and awaited significant development and guidance for its practice. Therefore, it is incumbent upon applicants to provide sufficient and enabling teachings within the specification for such therapeutic regimen. Although the instant specification provides a brief review of a potential therapeutic use of the claimed vector and data from ex vivo studies, it is not enabled for its full scope because the specification does not disclose whether the nucleic acids encompassed by the claims would function properly in vivo, any significant gene transfer in any target cells *in vivo*, or any therapeutic effects *in vivo*. In summary, the teachings and guidance present in the specification, as a whole, represent an initial investigation into the feasibility of the development of a useful means for executing gene

therapy in pluripotent precursor cells that awaits further development to the practical level.

Accordingly, in view of the quantity of experimentation necessary to determine the parameters for achieving *in vivo* gene expression in a significant population of the selected target cells at therapeutic levels, in particular for modifying pluripotent stem cells from any species, and the treatment of any and all diseases, the lack of direction or guidance provided by the specification as well as the absence of working examples with regard to *in vivo* gene transfer and therapeutic effects, and the breadth of the claims directed to the use of numerous combinations of therapeutic genes/tissue-specific promoters/vectors/expression regulatory elements, it would have required undue experimentation for one skilled in the art to make and/or use the claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-3, and 9-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 2 is vague and indefinite because of the claim recitation "native conditions" (lines 6 & 12). The specification fails to define the term, it is unclear the meaning of the term in the context of the claims, thus, the metes and bounds of the claims are uncertain.

Claim 9 recites the limitation "the marker gene" in line 10. There is insufficient antecedent basis for this limitation in the claim.

Claim 10 recites, "Expression cassette as claimed in claim 1 further comprising a second therapeutic gene". However, there is insufficient antecedent basis for the limitation in the claim because claim 1 does not recite a first therapeutic gene, and it is uncertain wihich element in claim 1 should be considered as therapeutic gene, thus the metes and bounds of the claims are unclear.

Claim 11 recites the limitation "the first and second therapeutic gene" in line 2. There is insufficient antecedent basis for this limitation in the claim. This is because neither claims 1, 2, or 10 recite a first therapeutic gene, nor claims 1 or 2 recite a second therapeutic gene.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-5, 12, and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by *Segre et al* (5,494,806).

The claims are drawn to an expression cassette and vector containing such cassette, the cassette comprising a tissue-specific promoter, at least one other

regulatory sequence, and a nucleic acid sequence encoding at least one non-immunogenic, cell surface receptor, wherein the receptor is not expressed naturally on the pluripotent progenitor cells, wherein the receptor has affinity for a ligand or an immunoglobulin molecule. The cassette may further comprise poly-cistronic gene controlling the coding sequence for a marker gene/therapeutic gene, preferably the coding sequence is for human.

Segre et al teach a nucleic acid encoding a human PTH receptor (SEQ ID No: 4), which is non-immunogenic for human, operably linked to a tissue-specific promoter (column 26, lines 5-20) contained in a vector comprising replication origins, selectable markers, signal sequences, and regulatory control sequences along with ribosome binding site sequence (poly-cistronic gene, column 20, lines 11-43), wherein the PTH is not expressed naturally on the pluripotent progenitor cells, and has affinity for a ligand (column 4, line 5) or an immunoglobulin molecule (abstract). Thus, *Segre et al* anticipate instant claims.

Claims 1, 2, 4, 5, 12, and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by *Reppert et al* (5,856,124).

Reppert et al teach a nucleic acid vector comprising a tissue-specific promoter and other control sequence (column 27, lines 43-48) operably linked to a coding sequence for human melatonin receptor (column 3, lines 29-50), which is not expressed naturally on the pluripotent progenitor cells, and has affinity for a ligand (column 4, lines

10-47) and an immunoglobulin molecule (column 28). Thus, *Reppert et al* anticipate instant claims.

Claims 1, 2, 4, 5, and 16 are rejected under 35 U.S.C. 102(b) as being anticipated by *Harlan et al* (5,718,883).

Harlan et al teach a vector (fig. 1 and abstract) comprising an expression cassette containing a tissue specific promoter (rat insulin promoter) operably linked to a nucleic acid encoding cell surface antigen B7 (column 4, line 37) and intron and polyA regulatory elements, wherein the B7 is not expressed naturally on the pluripotent progenitor cells, and has affinity for a ligand (CD28) or an immunoglobulin molecule (column 1, line 66). Thus, *Harlan et al* anticipate instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-6, 10, 12, 13, 15-17, and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24, IDS), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), in view of *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), and *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125).

Claims 1-6, 10, 12, 13, 15-17 are drawn to an expression cassette and vector containing such cassette, the cassette comprising a tissue-specific promoter, at least one other regulatory sequence, and a nucleic acid sequence encoding at least one non-immunogenic cell surface receptor that has binding affinity to a ligand or an antibody, preferably, the promoter is MLC-2v optionally with a CMV enhancer, and the receptor is surface antigen such as CD4 or shortened fragment thereof, e.g. the extracellular and transmembrane domain of CD4. In a preferred embodiment, the coding sequence is contained in a poly-cistronic gene comprises a marker gene and/or a therapeutic gene, preferably a human gene, wherein the construct further comprises a second therapeutic gene. Claim 32 is drawn to a method for genetic alteration of pluripotent precursor cells with the expression cassette.

Klug et al teach a method for genetic alteration of cultivated murine embryonic stem cells (pluripotent precursor cells) comprising transforming the ES cells with a vector comprising an expression cassette (abstract), wherein the cassette comprising a cardiomyocyte-specific promoter (α-cardiac myosin heavy chain promoter, MHC) operably linked to two marker gene, neo^r resistance gene and pGK hygromycin sequence, and a polyA regulatory element (paragraph bridging pages 216-7). *Klug et al* teach using such method for selecting cardiomyocytes from differentiating ES cells because the marker gene is driven by the MHC promoter, thus only expresses in differentiated cardiomyocytes. *Klug et al* teach that the method should be applicable to all ES-derived cell lineages, provided that suitable cell type-specific promoters are available. *Klug et al* also teach other cardiomyocyte-specific promoter such as MLC-2v

(right column, page 216). *Klug et al* do not teach a receptor in the expression cassette or a poly-cistronic gene cassette.

Gaines et al teach a poly-cistronic expression cassette comprising a nucleic acid encoding the extracellular and transmembrane domain of the human CD4 (receptor) and a luciferase marker gene operably linked to a dicistronic IRES, which mediates translational initiation. They transfect myeloid cells with the expression vector, and selecting for cells expressing CD4 surface marker by FACS. *Gaines et al* teach that the dicistronic construct is advantages over co-transfected or dual-promoter vectors because the expression could be coupled (mid-column, page 1), that cells transfected with pIRES-CD4t can be efficiently retrieved and analyzed quantitatively using commercially available techniques such as FACS and MCAS. They go on to teach that since CD4 is expressed in developing thymocytes and T lymphocytes, the marker can be used in a variety of cells and cell lines. *Gaines et al* use a CMV promoter/enhancer, do not teach a tissue specific promoter.

Griscelli et al teach an adenoviral vector comprising an expression cassette containing a MLC-2v promoter, which could specifically targeting the gene of interest to be expressed in cardiac muscle (abstract), and which is more specific than the MHC promoter (right column, page 1920). *Griscelli et al* teach that this is advantageous in specifically delivering a therapeutic cardiac gene (last sentence in Summary).

Wolfgang-M et al teach that when combining the MLC-2v promoter with the CMV enhancer, the expression of the marker gene luciferase increased two-fold and 15-fold *in vitro* and *in vivo* respectively.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al, Wolfgang-M et al, and Griscelli et al*, by combining the MHC promoter construct with the pIRES-CD4t construct and replacing the MHC with the MLC-2v/CMV promoter/enhancer, with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to make the modification because the combined construct offers more efficient transgene expression and additional means for cardiomyoctes selection, i.e. combining tissue-specific expression marker with cell sorting technology. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), as applied to Claims 1-6, 10, 12, 13, 15-17, and 32 above, and further in view of *Cheng et al* (Gene Ther 1997;4:1013-22).

Claim 9 is drawn to a marker gene that is green fluorescent protein. The combined teachings of *Klug et al, Gaines et al, Wolfgang-M et al,* and *Griscelli et al* teach a luciferase but not a GFP as the reporter gene.

However, before the instant effective filing date, *Cheng et al* teach that GFP is an effective vital marker to quantify viral vector mediated gene transfer into hematopoietic

and stem/progenitor cells, and could be used for monitor gene expression during their subsequent cell lineage determinations (Abstract).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al Wolfgang-M et al, and Griscelli et al*, by replacing luciferase with GFP in the MLC-2v-pIRES-CD4t construct with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because it is within the knowledge of the skill to select a well-known marker gene of interest in cell tagging. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 11 and 14 (a) (b) are rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), as applied to Claims 1-6, 10, 12, 13, 15-17, and 32 above, and further in view of *Mack et al* (J Thorac Cardiovasc Surg 1998;115:168-76).

Claim 11 is drawn to using angiogenesis factors such as VEGF as therapeutic genes. Claim 14 (a) (b) is drawn to a preferred expression cassette comprising MLC-2v promoter, CMV enhancer, CD4 extracellular and transmembrane domains, IRES, and a angiogenesis factor as the therapeutic gene. The combined teaches of *Klug et al*,

Gaines et al, Wolfgang-M et al, and *Griscelli et al* do not specify that the therapeutic gene is an angiogenesis factor such as VEGF.

However, before the instant effective filing date, *Mack et al* teach that a potent angiogenic mediator such as VEGF could be delivered genetically to ischemic region of the myocardium to enhance collateral vessel formation and improve regional perfusion and function.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al, Wolfgang-M et al, and Griscelli et al*, by including a nucleic acid coding for an angiogeneic factor in the MLC-2v-pIRES-CD4t construct with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because it provides additional means for repairing damaged myocardium with both therapeutic cells and angiogenic factors. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 7 and 8 are rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), and *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), as applied to Claims 1-6, 10, 12, 13, 15-17, and 32 above, and further in view of *Graham et al* (US 6,080,569).

Claims 7 and 8 are drawn to the expression cassette comprising a tissue-specific promoter, and a surface receptor further comprising a reversibly integrated resistance gene flanked by loxP sequences. The combined teachings of *Klug et al*, *Gaines et al, Wolfgang-M et al,* and *Griscelli et al* do not teach such a reversibly integrated resistance gene.

However, before the instant effective filing date, *Gramham et al* teach that an adenoviral expression construct (figures 3 & 4) comprising a neomycin resistance gene along with the viral packaging signal flanked by two loxP sites, and upon infection of cells that express the Cre recombinase, the neo^r gene and packaging signal would be excised. They illustrated with the example how such system could be used to include or delete certain elements of the construct as needed (fig. 10).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al, Wolfgang-M et al, Griscelli et al*, and *Gramham et al* by including a reversible antibiotic resistance gene in the MLC-2v-pIRES-CD4t construct with a reasonable expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention because it is known in the art that the resistance gene is desirable for selection, and undesirable to be present in a therapeutic agent, such as therapeutic cells. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claim 14 (c) (d) is rejected under 35 U.S.C. 103(a) as being unpatentable over *Klug et al* (J Clin Invest 1996;98:216-24), *Gaines et al* (IDS, Biotechniques 1999;26:683-8), *Wolfgang-M et al* (J Mol Cell Cardiol 1997;29(5):A125), *Griscelli et al* (Hum Gene Ther 1998;9:1919-28), and *Graham et al* (US 6,080,569), as applied to Claims 1-8, 10, 12, 13, 15-17, and 32 above, and further in view of *Gainer et al* (Transplant 1998;66:194-9), and *Lallemand et al* (Transgenic Res 1998;7:105-12).

Claim 14 (c) (d) is drawn to a preferred expression cassette comprising MLC-2v promoter, CMV enhancer, CD4 extracellular and transmembrane domains, IRES, and a angiogenesis factor, LoxP, PGK promoter, resistance gene, LoxP, PGK promoter, CTLA4-Ig fusion protein (claim 14 c & d). The combined teaches of *Klug et al*, *Gaines et al, Griscelli et al,* and *Graham et al* do not teach such a CTLA4-Ig or a PGK promoter.

However, before the instant effective filing date, *Gainer et al* teach CTLA4 can suppress graft rejection response and prolong the survival of transplanted islet cells (CTLA4 transfected, abstract); and *Lallemand et al* teach a PGK promoter used in conjunction with the LoxP construct. The PGK promoter activates very early in the pluripotent progenitor cells and displays ubiquitous expression (2nd paragraph, page 106, and page 109), thus, suitable for genetic modification of stem cells.

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Klug et al, Gaines et al, Wolfgang-M et al, Griscelli et al,* and *Graham et al*, by including a CTLA4 gene and PGK promoter as taught by *Gainer et al* and *Lallemand et al* in the MLC-2v-pIRES-CD4t-loxp construct for manipulation of pluripotent progenitor cells with a reasonable

expectation of success. The ordinary skilled artisan would have been motivated to modify the claimed invention when using the pluripotent stem cells as therapeutic cells for transplantation because including a CTLA4 in the construct would enhance the survival of cells after transplantation and including a PGK promoter would made the transgene expressed early on. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Q. Janice Li whose telephone number is 703-308-7942. The examiner can normally be reached on 8:30 am - 5 p.m., Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah J. Reynolds can be reached on 703-305-4051. The fax numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of formal matters can be directed to the patent analyst, Dianiece Jacobs, whose telephone number is (703) 305-3388.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-1235. The faxing of such papers must conform to the notice published in the Official Gazette 1096 OG 30 (November 15, 1989).

Q. Janice Li
Examiner
Art Unit 1632

QJL
July 14, 2003